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Automated Single-Slide Staining Device

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An automated single-slide staining device is described. Smears gram stained by the automatic device were equal in quality to the manual method.

A frequently performed operation in a microbiology laboratory is the Gram stain. It would be highly desirable to automate this procedure in order to free the operator for other work and to remove certain subjective evaluations such as the decolorization step. Two automated Gram stain machines have been developed (1, 2). Both units operate on a "batch" system where slides are automatically transported through troughs containing staining solutions. The obvious drawback to such a system is the possibility of contamination, either through the staining solutions themselves or via transfer from previously processed slides.

We describe here an automated staining device based on the single-slide principle. This unit has all the features of automation with a lower probability of contamination than batch systems. In addition to the Gram stain, this unit is also flexible enough to accommodate other types of staining procedures used in the microbiology laboratory.

A front and back view of the automatic staining device is shown in Fig. 1. Four glass containers, coated on the outside with black enamel paint to prevent light-induced stain deterioration, contained the following stains and reagents: gentian violet (Weigert no. 1), Gram iodine solution, decolorizer consisting of two parts 95% ethyl alcohol and one part acetone, and 1% aqueous safranin. All stains were purchased from the Fisher Scientific Co. (Fair Lawn, N.J.). A glass container held distilled water for the rinse cycles. Located below each glass container was a timer-actuated solenoid (B2DA102B, Skinner Electric Valve Co., New Britain, Conn.) to control dispensing of tube contents in proper sequence and for the required time to achieve optimum staining. The five solenoid valves were controlled by a timing motor (11C-NSY, Minarik Electric Co., Los Angeles, Calif.) and five cams attached to the motor's shaft. The cams actuated microswitches to control each solenoid and length of staining time was accomplished by varying the cam slot. The amount of stain or reagent delivered was controlled by regulating the stopcock located directly below each solenoid and the amount delivered was not influenced by reservoir fill. Glass tubing from each stopcock was configured to a position over a glass slide retained in a glass funnel and tubing from the funnel to a sink permitted disposal of used stains, reagents, and water. The device was actuated by pushing the start button. In a separate mode, provisions were made for manually flushing the entire system by opening the stopcocks located below each stain reservoir and operating a six position selector switch (419 DXD, Globe Union Inc., Milwaukee, Wisc.) which actuated each solenoid separately.

The time to complete 1 Gram stain cycle was 4.80 min and the times required for each stage of the staining process are shown in Table 1.

Twenty smears each of five gram-negative and five gram-positive cultures were prepared and heat fixed according to accepted procedures; 10 smears were Gram stained by the automatic device and 10 by a manual method. The staining times were the same for both procedures. In addition, 10 smears were prepared, each consisting of a mixture of a gram-positive with a gram-negative organism, and these smears were stained by either the automatic or manual method.

Although no attempt was made to quantitatively grade the stained preparations, as described by Drew et al. (2), subjective evaluations by three experienced observers of all slides indicated no differences in the quality of the smears stained by the automatic versus the manual method. In addition, no untoward effects were observed, either in the mechanical operation of the automatic machine, or quality of stained smears, when the procedures were conducted on 5 different days over a period of 30 days.

As in the case of batch techniques (1, 2), the major advantages of the automatic stain procedure described in this report consisted of (i) freeing the operator for other work in the laboratory, and (ii) elimination of any subjective

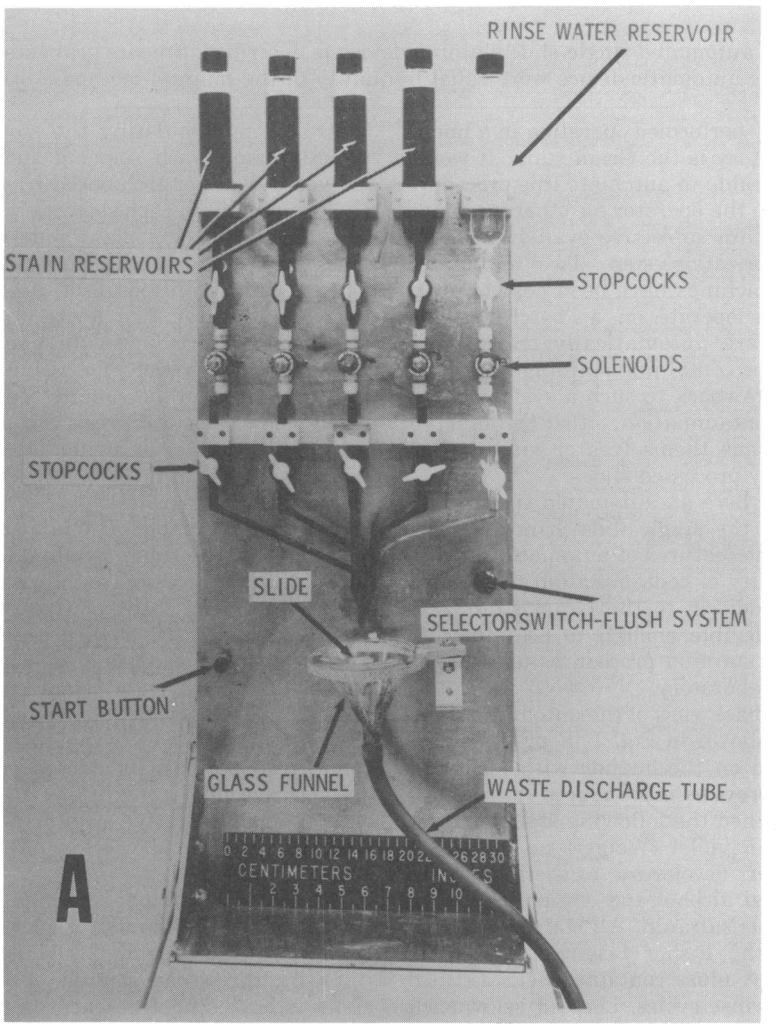

FIG. 1. *Front (A) and back (B) views of the automated single-slide staining device.*

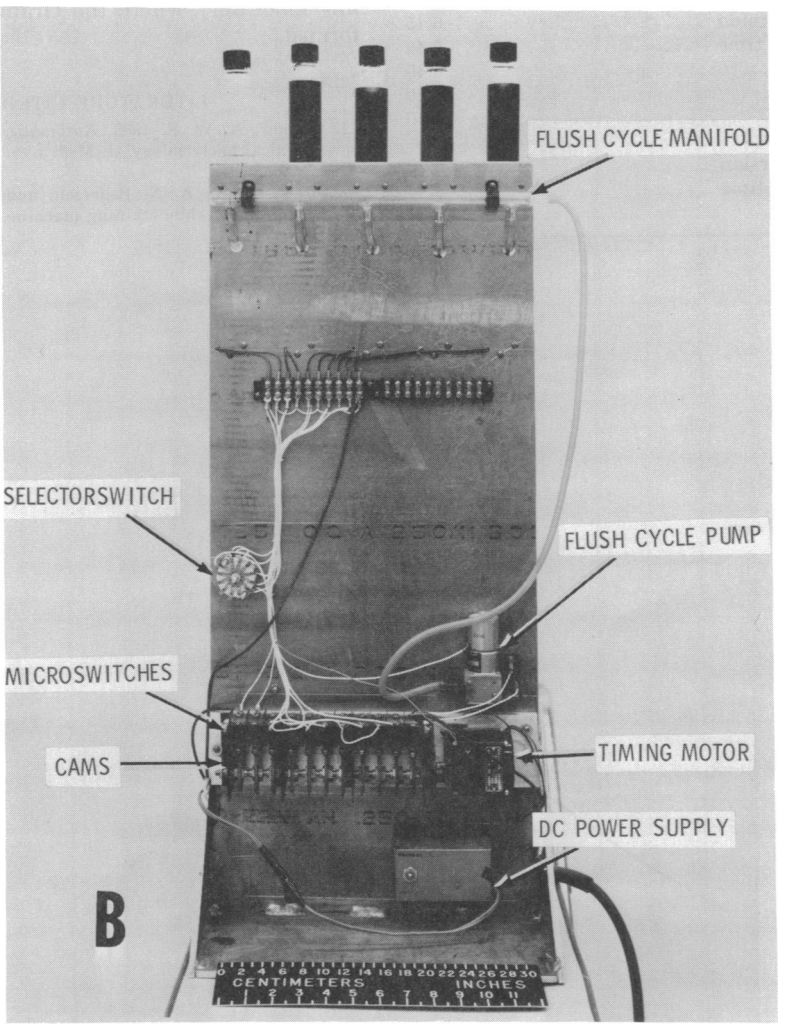
FLUSH CYCLE MANIFOLD
SELECTORSWITCH
FLUSH CYCLE PUMP
MICROSWITCHES
CAMS
TIMING MOTOR
DC POWER SUPPLY
B
CENTIMETERS
INCHES

FIG. 1B

TABLE 1. *Automated gram stain cycle*

Stage	Time (min)
Gentian violet	
Stain dispersion	0.25
Total stain time	1.00
Rinse cycle	0.40
Iodine	
Stain dispersion	0.15
Total stain time	0.40
Rinse cycle	0.40
Decolorizer	0.10
Rinse cycle	0.40
Safranin	
Stain dispersion	0.40
Total stain time	0.90
Rinse cycle	0.40

evaluations as to length of staining or decolorizing time. Such a procedure ensured uniform and repeatable staining of smears, free of any operator involvement. On the other hand, in contrast to batch methods, this device eliminated any possibility of cross contamination from other slides. This device could be useful in any laboratory where the Gram stain is performed.

LITERATURE CITED

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